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DEVELOPING FIELD SCREENING TECHNIQUES FOR STEMPHYLIUM BLIGHT IN LENTIL

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Developing field screening techniques for stemphylium blight in lentil

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Abstract / Summary

Stemphylium botryosum causes leaf blight on lentil that can result in large-scale defoliation of plants. This pathogen has been observed increasingly in lentil fields in Saskatchewan and on seed tested for infection with *A. lentis* and other common lentil pathogens. Experimentation in the field and the establishment of stemphylium blight nurseries has been hampered by lack of good inoculation and incubation protocols. Four different cover materials for low tunnels commonly used in horticulture were evaluated for the establishment of stemphylium blight in lentil plots. Among the materials tested were perforated white polyethylene that filters 26% of the incoming light resulting in cooler temperatures, perforated clear polyethylene, providing warm conditions, perforated green polyethylene that filters out 41% of the incoming light resulting in cooler conditions and Novagryl woven polyester that filters approximately 20% of the incoming light, is highly perforate resulting in cool conditions. Stemphylium blight severity ranged from 45 to 95% in 15 lentil cultivars / breeding lines which is considered a very good for selection purposes. It is noteworthy to mention that some cultivars / breeding lines did not react to the pathogen as expected and may need more testing to confirm resistance levels. All cover materials were equally good at promoting stemphylium blight development in lentil cultivars / breeding lines. However, the majority of cultivars / breeding lines had higher yields under the perforated green polyethylene material, which also resulted in slightly lower temperatures. TSW as well as seed size appeared to be negatively affected by the white cover material. It is not known whether this is a temperature or light effect, or a combination of both. Seed infection rates were lowest under Novagryl. The perforated green and possible the Novagryl material thus warrant further large scale testing in replicated trials, to determine whether this approach can be used in warmer growing seasons.

Introduction

Stemphylium botryosum causes leaf blight on lentil that can result in large-scale defoliation of plants (Allen & Lenne, 1998). The fungus has been a serious pathogen in Bangladesh for many years where yield losses of 62 % and more have been experienced due to the disease (Allan 1998). In recent years, this disease has been observed increasingly in lentil fields in Saskatchewan and the pathogen has been recovered at higher rates from seed tested for infection with *A. lentis* and other common lentil pathogens ((Morrall et al 2006). *Stemphylium botryosum* is a poorly researched fungus and very little information is available on the pathogen on lentil in a semi-arid climate like that of Saskatchewan. Large-scale screening has been seriously hampered by lack of knowledge of the biology of the pathogen. As a consequence of this, information is sketchy on optimal conditions for serious outbreaks of stemphylium blight, yield loss caused by the disease and appropriate disease management strategies. A 1-year trial funded through BASF suggested that Headline had efficacy against the pathogen, and on-farm experiments indicated that seed size may be affected by stemphylium blight (Banniza, unpublished data), but all trials suffered from suboptimal experimental conditions due to knowledge gaps. As a result, it is currently not possible to assess whether fungicide applications specifically for the control of stemphylium blight are warranted. To address several of these gaps, more data on the time of appearance of the disease and its severity in combination with weather data need to be collected to identify factors that lead to epidemic outbreaks of stemphylium blight. In order to determine yield loss and disease management strategies through systematic field experiments, as well as initiate routine

screening of breeding material in the lentil breeding program, a field screening protocol has to be developed.
In contrast to *Ascochyta lentis* and *Colletotrichum truncatum*, which are the main fungal pathogens in lentil, *Stemphylium botryosum* is an air-borne pathogen that does not require rain for spore dispersal. Moisture on lentil leaves, whether through dew, rain or possibly very high humidity, is, however, required for the spores to germinate and infect the lentil tissue. Field experimentation with this pathogen, whether it is for resistance screening or for disease management purposes, thus pose two problems, i) that inoculated field experiments can be a source of significant spore clouds that could be blown into other, unrelated lentil trials, and ii) that inoculation may be unsuccessful because conditions remain too dry for these spores to infect.
This pilot project was initiated to evaluate the use of low tunnels commonly used in vegetable production in combination with a misting irrigation system, as a potential method to conduct field experiments on stemphylium blight. This approach could potentially restrict the spread of spores while promoting humid conditions conducive for spore germination and infection. As spore production for the purpose of large scale inoculation has been difficult, infected spreader plants were integrated into the seeded plots.

Method

Microplots of 1 m length with 3 rows at 25.4 cm row spacing were established into summer fallow on research land at the University of Saskatchewan on May 28, 2008. The area had been treated with Edge at 27.92 kg ha^{-1} in the fall of 2008. Fifteen cultivars and breeding lines (referred to as cultivars hereafter) including those with assumed reactions to stemphylium blight (R = partial resistance, S = susceptible) were seeded in four rows of micro-plots with a Fabro disc opener minimum tillage seeder. These cultivars / breeding lines were:

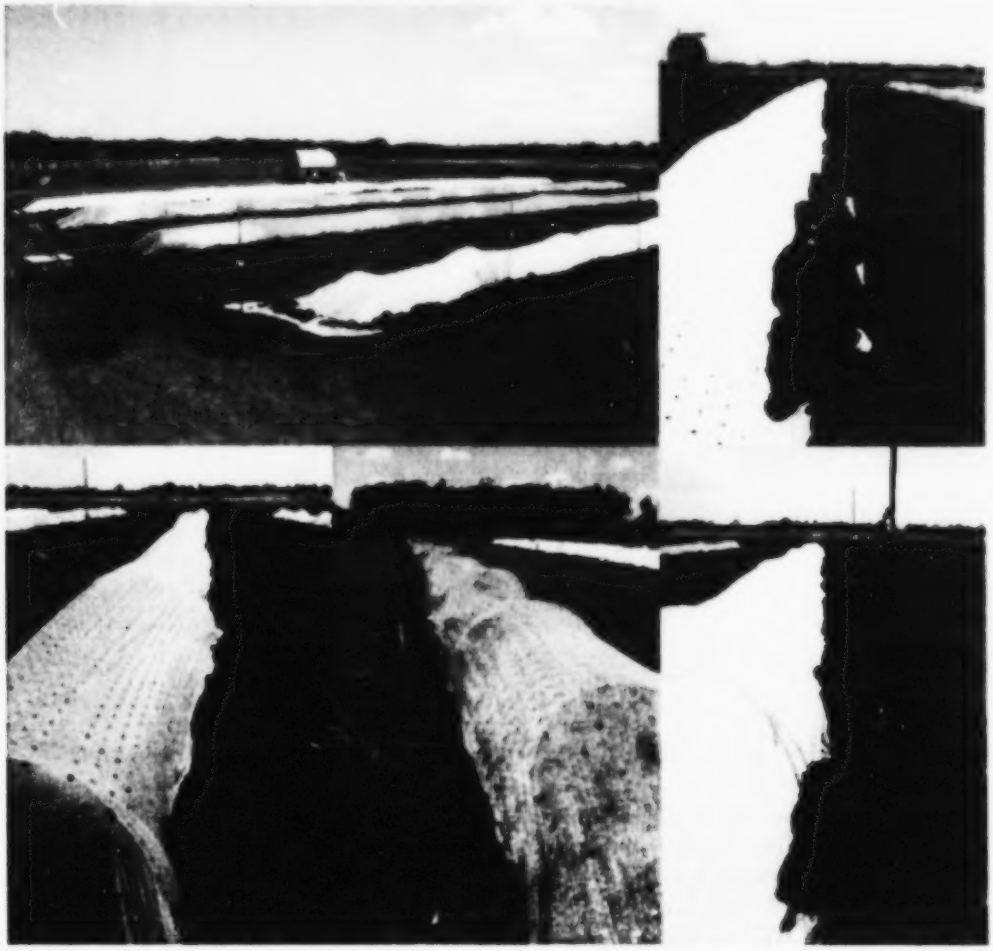
1921T-11 (R), CDC Impala, 1194-3 (R), CDC Vantage (S), CDC Milestone (S), A9 (S), Pardina (S), 1894T-1 (R), CDC Impact, Precoz (ILL 4605) (R), CDC Red Rider (1308M-7) (R), CDC Robin (S), Barimasur-4 (R), CDC Blaze (R), MP52-1 (R).

Spreader plants were produced in the phytotron in 56 one-gallon pots at 6 Eston plants per pot. Plants were maintained at 23°C day/18°C night and 16 hrs light/8 hrs dark. Four-week old plants were spray-inoculated with a mycelium suspension (3g mycelium in 1000 mL H_2O; 20 mL suspension/pot) of *S. botryosum* isolate SB-19 on June 26, 2009 and incubated in a mist chamber for 72 hours. Plants were kept in a tent to prevent drying and misted by hand on a weekly basis to encourage disease development.

On July 17, 2009, three weeks after inoculation, one pot with spreader plants was transplanted in-between each of two adjacent micro-plots at the field site. Transplants were irrigated to promote establishment between the microplots. Each lentil strip was then equipped with temperature sensors and metal hoops placed at 1.8 m distance. Instead of using one material for covering the rows, it was decided to evaluate four different materials even thought his meant not having a replicated trial (Figure1). The four materials selected have different effects on the microclimate, which is very important as overheating under the cover could potentially be fatal to lentil plants. The materials assessed were:

a) Perforated white polyethylene (PE) that filters 26% of the incoming light resulting in cooler temperatures;
b) Perforated clear PE providing warm conditions;
c) Perforated green PE that filters out 41% of the incoming light (with potential effects on growth of the plants) resulting in cooler conditions;
d) Novagryl woven polyester that filters approximately 20% of the incoming light, is highly perforate resulting in cool conditions.

Figure 1. Clockwise from top left: Rows of lentil microplots in low tunnels with different cover material to promote the development of stemphylium blight. Tunnel with perforated white polyethylene cover material. Tunnel with Novagryl woven polyester cover material. Tunnel with perforated clear polyethylene cover material. Tunnel with perforated green polyethylene cover material.



A misting irrigation system was established between rows with sprinklers covering the rows. Misting irrigation was scheduled daily at 2-hour interval for 5 min between 7am to 1 pm, at 1-hour intervals for 5 min between 1pm-4pm, and at 2-hour intervals for 5 min from 4pm-8pm.

Rows were monitored for plant and disease development on a weekly basis. Row cover was removed after 5 weeks on August 24, 2009 for disease scoring. An incremental scale where 0=0%, 1=1-10%, 2=11-20%, 3=21-30%, 4=31-40%, 5=41-50%, 6=51-60%, 7=61-70%, 8=71-80%, 9=81-90%, and 10=91-100% was used to assess severity of stemphylium blight.

Field plots were harvested and bagged individually on September 24, 2009, and samples were then placed on a drying bench for two days. Threshing was done manually by feeding plants into a threshing machine, and seeds were cleaned using a blower and several seed screens. Seed yield was obtained by weighing the cleaned seeds of each plot. Thousand seed weight (TSW) was determined based on a 200 seeds subsample. Sizing was done using screens with round holes of 15/64, 14/64, 13/64, 12/64, 11/64, 10/64, 9/64, and 8/64 (in fractions of an inch) diameters, and the weight of each size sample was recorded.

Disease testing consisted of plating 100 seeds per plot onto sterile large Petri dishes (10 per dish) containing Potato Dextrose Agar (29.25g Potato Dextrose Agar powder, 750ml distilled water, 100 mg L^{-1} Streptomycin added after autoclaving). After seven days the seeds were visually inspected for *Stemphylium*, *Botrytis*, *Sclerotinia*, *Fusarium*, *Penicillium*, and *Colletotrichum*.

Results

Stemphylium blight severity ranged from 45 to 95% in the tunnels, which is optimal for selection of resistant germplasm (Table 1, Figure 2). Management of air temperature in the tunnels was considered to be a critical aspect determining whether it would be feasible to use tunnels for lentil disease screening. As expected, temperatures in the tunnels were significantly higher than outside air temperature (Figure 3). The perforated clear polyethylene material caused temperatures to rise higher than the other materials, and exceeded 40°C in on July 18, which was randomly selected to exemplify temperature curves. The other 3 materials performed similarly, but even here it was not uncommon to have temperatures between 30 and 40°C in the afternoon.

Stemphylium blight severity reached over 90% in some of the plots. Disease reaction was not as expected in all lentil cultivars (Table 1). For example, Barimasur-4 is a stemphylium blight resistant cultivar from Bangladesh that was highly susceptible in our trial. Disease reactions among lentil cultivars were not very consistent in response to the different row cover material. Highest consistency was found between perforated green PE and Novagryl (R = 0.8327), under which disease severity was also somewhat lower.

Table 1: Stemphylium bight severity (%) in field microplots of lentil cultivars and breeding lines covered with different row tunnel material. Green highlighted cultivars / breeding lines were expected to show a partially resistant, red a susceptible reaction.

Cultivar/Breeding Line	Perforated white PE	Perforated clear PE	Perforated green PE	Novagryl	Mean
1194-3	75	75	65	55	67.5
CDC Red Rider	45	65	65	65	60
1894T-1	55	65	75	75	67.5
1921T-11	55	65	65	45	57.5
A9	65	45	45	45	50
Barimasur-4	95	85	95	95	92.5
CDC Blaze	55	65	45	45	52.5
CDC Robin	45	75	65	75	65
CDC Vantage	65	75	45	45	57.5
CDC Impact	65	75	65	45	62.5
CDC Impala	75	65	55	65	65
CDC Milestone	55	75	85	75	72.5
MP52-1	65	55	45	15	45
Pardina	95	95	95	95	95
Precoz(ILL 4605)	65	85	55	65	67.5
Mean	65	71	64	60	

Figure 2. Examples of differential stemphylium blight development with susceptible (brown) accessions and partially resistant (green) accessions.

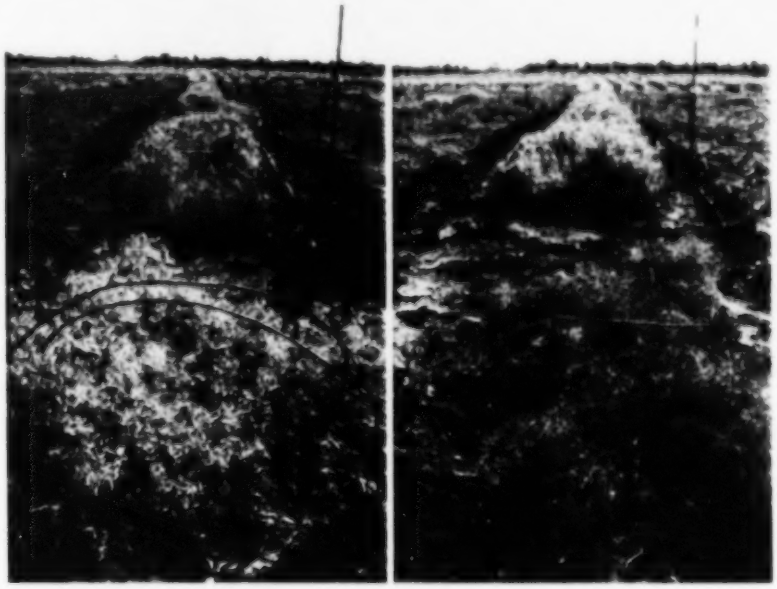
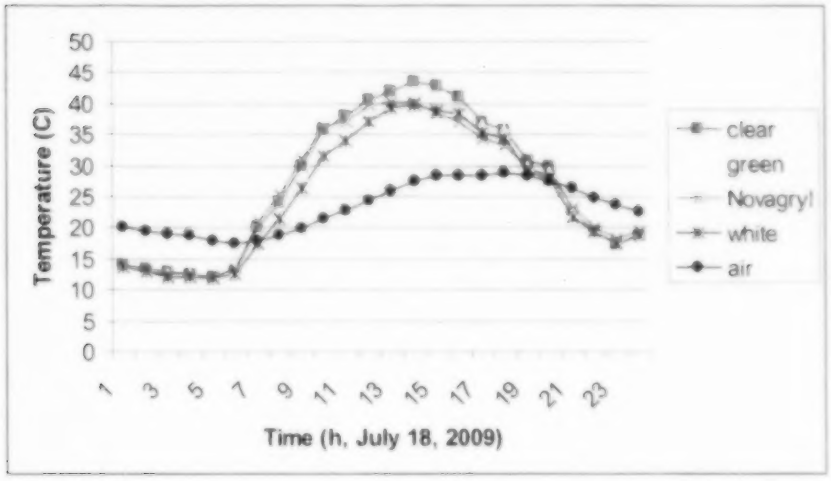


Figure 3. Temperature curve in 4 low tunnels covered with perforated clear (clear), green (green), and white (white) polyethylene or Novagryl woven polyester.



For the majority of lentil cultivars, seed yield was highest in tunnels covered with the perforated green polyethylene (Table 2). The exceptions to this were CDC Red Rider and 1921T-11 for which yields were similar irrespective of row cover material, Barimasur-4 for which only perforated white PE depressed yields, and CDC Robin for which yield was highest under perforated white PE and Novagryl.

Table 2: Lentil seed yield from microplots grown in low tunnels with 4 different cover materials.

Cultivar/Breeding line	Perforated white PE	Perforated clear PE	Perforated green PE	Novagryl	Mean
1194-3	651	597	1243	1496	997
CDC Red Rider	416	303	469	391	395
1894T-1	804	785	395	333	579
1921T-11	75	171	87	70	100
A9	175	354	654	297	370
Barimasur-4	59	390	344	356	287
CDC Blaze	270	411	1184	2175	1010
CDC Robin	1165	612	421	1125	831
CDC Vantage	220	252	907	1891	818
CDC Impact	444	196	1259	1832	932
CDC Impala	576	1033	1154	671	858
CDC Milestone	490	403	785	1230	727
MP52-1	648	919	1135	2965	1417
Pardina	142	209	341	529	305
Precoz(ILL 4605)	241	105	951	572	468
Mean	425	449	755	1062	

Thousand seed weight was consistently lower under perforated white PE, but varied noticeably under the other cover materials (Table 3).

Table 3. Thousand seed weight (g) of lentil cultivars / breeding lines from microplots grown in low tunnels with 4 different cover materials.

Cultivar/Breeding Line	Perforated white PE	Perforated clear PE	Perforated green PE	Novagryl	Mean
1194-3	23	27	31	24	26
CDC Red Rider	31	28	29	32	30
1894T-1	24	22	17	21	21
1921T-11	33	35	21	27	29
A9	21	32	32	31	29
Barimasur-4	15	34	22	22	23
CDC Blaze	24	26	29	30	27
CDC Robin	24	22	22	23	23
CDC Vantage	32	40	32	39	36
CDC Impact	24	27	29	26	27
CDC Impala	22	24	26	22	23
CDC Milestone	27	31	26	29	28
MP52-1	35	38	37	41	38
Pardina	21	26	21	25	23
Precoz(ILL 4605)	37	33	34	43	37
Mean	26	30	27	29	

Seed sizing showed that most seeds were in the 11/64, 10/64 and 9/64 inch size fractions, so for ease of reading and interpretation, we only present the results of these size classes.

Approximately half of the lentil cultivars had most seeds in the 10/64 and 9/64 fractions when grown under perforated white PE (Tables 5 & 6), but not in the larger fraction, suggesting that this material may negatively affect seed size. The majority of larger seeds (11/64) developed on plants grown under clear PE.

Table 4. Percentage seed in the 11/64 inch size fraction of cultivars / breeding lines from microplots grown in low tunnels with 4 different cover materials.

Cultivar/Breeding Line	Perforated white PE	Perforated clear PE	Perforated green PE	Novagryl	Mean
1194-3	7	12	10	2	8
CDC Red Rider	26	21	35	31	28
1894T-1	1	6	2	2	3
1921T-11	25	21	15	12	18
A9	11	23	28	23	21
Barimasur-4	7	14	4	4	7
CDC Blaze	8	16	21	21	17
CDC Robin	1	2	0	1	1
CDC Vantage	25	24	27	24	25
CDC Impact	10	18	16	13	14
CDC Impala	3	8	6	3	5
CDC Milestone	24	29	12	18	21
MP52-1	36	31	36	46	37
Pardina	11	20	8	18	14
Precoz(ILL 4605)	23	15	14	15	17
Mean	15	17	16	16	

Table 5. Percentage seed in the 10/64 inch size fraction of cultivars / breeding lines from microplots grown in low tunnels with 4 different cover materials.

Cultivar/Breeding Line	Perforated white PE	Perforated clear PE	Perforated green PE	Novagryl	Mean
1194-3	29	28	29	44	33
CDC Red Rider	18	16	13	12	15
1894T-1	37	32	25	28	31
1921T-11	8	8	29	11	14
A9	28	20	17	20	21
Barimasur-4	22	23	30	35	27
CDC Blaze	28	24	22	21	24
CDC Robin	42	33	37	33	36
CDC Vantage	14	8	8	4	9
CDC Impact	28	20	22	28	24
CDC Impala	32	30	31	42	34
CDC Milestone	20	16	29	27	23
MP52-1	14	12	14	7	12
Pardina	26	22	28	26	26
Precoz(ILL 4605)	8	7	8	4	7
Mean	24	20	23	23	

Table 6. Percentage seed in the 9/64 inch size fraction of cultivars / breeding lines from microplots grown in low tunnels with 4 different cover materials.

Cultivar/Breeding Line	Perforated white PE	Perforated clear PE	Perforated green PE	Novagryl	Mean
1194-3	17	14	11	18	15
CDC Red Rider	9	11	6	8	8
1894T-1	33	19	30	29	28
1921T-11	4	5	15	19	11
A9	20	13	8	9	12
Barimasur-4	23	10	27	25	22
CDC Blaze	21	15	11	13	15
CDC Robin	32	24	33	38	32
CDC Vantage	8	4	5	2	5
CDC Impact	20	18	12	14	16
CDC Impala	25	19	19	26	22
CDC Milestone	14	10	18	13	14
MP52-1	7	5	6	2	5
Pardina	23	15	26	14	19
Precoz(ILL 4605)	4	5	4	2	4
Mean	17	12	16	15	

Seed infection with *S. botryosum* was low, so an evaluation of different cover materials was not possible for this disease. Infection by *Botrytis* sp. was highest among all pathogens recovered and revealed that lowest infection levels were under Novagryl woven polyester whereas it varied a lot under the other materials (Table 7).

Table 7. Seed infection rates with various fungal pathogens of lentils seeds of cultivars / breeding lines from microplots grown in low tunnels with 4 different cover materials (1: Perforated white polyethylene, 2: Perforated clear polyethylene, 3: Perforated green polyethylene, 4: Novagryl woven polyester).

Cultivar/Breeding Line	Stemphylium				Botrytis				Sclerotinia				Fusarium				Penicillium				Anthracnose			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1194-3	0	0	0	0	10	10	10	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
CDC Red Rider	0	0	0	0	100	10	0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0	0
1894T-1	0	0	0	0	0	0	80	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0
1921T-11	0	0	0	0	0	100	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A9	0	0	0	0	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0
Barimasur-4	0	10	0	10	100	0	10	30	0	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CDC Blaze	0	0	0	0	80	100	0	20	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0
CDC Robin	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	10	0
CDC Vantage	0	0	0	0	80	40	40	20	0	0	10	0	0	0	0	0	0	0	0	10	10	10	0	0
CDC Impact	0	0	0	0	0	100	100	0	0	20	0	0	0	0	0	0	10	0	0	0	10	0	0	0
CDC Impala	10	0	0	0	0	90	0	0	0	30	0	0	0	0	0	0	0	0	0	10	0	0	0	0
CDC Milestone	0	0	0	0	20	40	90	60	0	20	0	0	0	0	0	0	0	0	0	10	10	0	0	0
MP52-1	0	10	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	30	0	0	0	0	0	0
Pardina	0	20	20	0	20	0	0	10	0	0	0	0	0	10	0	20	0	0	0	10	0	0	0	0
Precoz(ILL 4605)	0	0	0	10	0	10	100	0	0	10	0	0	10	0	0	10	10	0	0	0	10	0	0	0
Mean	0.7	4	1.3	1.3	33	33	31	9.3	0.7	8	2	0	0.7	2.7	0	2.7	2	2.7	0	2.7	2.7	0.7	2	0

Conclusions and Recommendations

Four different cover materials for low tunnels were evaluated for the establishment of stemphylium blight in lentil plots. Among the materials tested were perforated white polyethylene that filters 26% of the incoming light resulting in cooler temperatures, perforated clear polyethylene, providing warm conditions, perforated green polyethylene that filters out 41% of the incoming light resulting in cooler conditions and Novagryl woven polyester that filters approximately 20% of the incoming light, is highly perforate resulting in cool conditions. Stemphylium blight severity ranged from 45 to 95% in 15 lentil cultivars / breeding lines. None of the materials consistently favoured stemphylium blight development in all cultivars / breeding lines. It is interesting to note that some cultivars / breeding lines did not react to the pathogen as expected and may need more testing to confirm resistance levels. The majority of cultivars / breeding lines had higher yields under the perforated green polyethylene material, which also resulted in slightly lower temperatures. TSW as well as seed size appeared to be negatively affected by the white cover material. Seed infection with *S. botryosum* was very low, but infection rates with *Botrytis* were lowest under the Novagryl material.

The perforated green and possible the Novagryl material warrant further large scale testing in replicated trials to determine whether they are useful even under warmer conditions than 2009. We believe that this may be the case. In 2009, cold conditions in the spring resulted in a delay in seeding and slow plant development. As a result, microplots were covered with the low tunnels relatively late. We anticipate that tunnels would usually be used earlier during the growing season thus possibly avoiding major problems with overheating inside the tunnels.

Spreader plants worked well in this pilot study, but would be too expensive and cumbersome for large scale screening or experimentation. We believe that it will be necessary to develop a protocol for the mass production of spores to be used as spray inoculum in the field. Such a protocol has been successfully developed for the related genus *Alternaria* in the Biobased platform research group of AAFC in Saskatoon. We intend to explore opportunities with them to develop such a protocol for *Stemphylium botryosum*.

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Other
None